

The association between serum lipid ratios and insulin resistance among subjects with impaired glucose tolerance in Pakistan

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Abstract

The aim of this study was to find the association between serum lipid ratios and insulin resistance among subjects with impaired glucose tolerance in Pakistan. It was also sought how effective was FBG to identify IGT subjects in context of using FBG as a screening tool for primary prevention.

Research design and methods:

Cross sectional analysis was conducted by utilizing the data from primary prevention project conducted within collaboration of Baqai Institute of Diabetology and Endocrinology (BIDE), Diabetic Association of Pakistan (DAP) and University of Oslo (UiO). Data analysis was divided into two section; the screening data, comprises of OGTT values of 1565 study participants after being short listed by using risk assessment form during the initial phase of project; and the baseline data, that constituted the anthropometric and biochemical variables of 242 subjects, included on basis of availability of all the required variables.

Results:

The prevalence of IFG, combined IFG-IGT and isolated IGT was found to be 10.9%, 11.3% and 12.1% respectively. The specificity and sensitivity of fasting blood glucose to diagnose IGT was 48.6% and 82.6%, while the positive predictive value was 50.7% and negative predictive value was found 81.1%. Data analysis at baseline showed no difference between mean ages and gender of combined IFG-IGT and isolated IGT group, however waist to hip ratio was significantly higher ($p < 0.05$) in combined IFG-IGT group. Also, the levels of 2-hr blood glucose and serum triglycerides were higher in combined IFG-IGT group while fasting insulin levels were higher in isolated IGT group. All the lipid ratios (TC/HDL, TG/HDL and HDL/HDL) were found significantly correlated with fasting insulin levels and HOMA-IR in isolated IGT group. The AUC for TG/HDL ratio was 0.707 against the 75th percentile of fasting insulin level and HOMA-IR, while the cut off level >3.36 of TG/HDL ratio was found to have 65.5% sensitivity and 70% specificity with respect to the cut off of insulin resistance

Conclusion:

We have found significant correlation between serum lipid ratio and surrogate markers of insulin resistance in isolated IGT group. Due to the weak predictability of fasting blood glucose to diagnose IGT status, it is therefore suggested to use better option for the diagnosis of IGT in clinical practice. However, serum lipid ratios could be a potential option if used in conjunction with fasting blood glucose to identify subjects with isolated IGT who are found significantly more insulin resistant, and thus, can be more benefited by primary prevention of diabetes.

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List of Abbreviation

ADA	American Diabetes Association
BMI	Body mass index
Combined IFG-IGT	Combined impaired fasting glucose and impaired glucose tolerance
CVD	Cardiovascular diseases
FBG	fasting blood glucose
FFA	Free fatty acids
HbA1C	Hemoglobin A 1 C
HDL	High density lipoproteins
HOMA-IR	Homeostasis model assessment – Insulin resistance
IFG	Impaired Fasting Glucose
IGT	Impaired glucose tolerance
I-IFG	Isolated impaired fasting glucose
I-IGT	Isolated impaired glucose tolerance
IR	Insulin resistance
LDL	Low density lipoproteins
LDL/HDL	Low density lipoproteins to high density lipoprotein ratio
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
2-hr OGTT	Two hour oral glucose tolerance test
TC	Total cholesterol
TC/HDL	Total cholesterol to high density lipoprotein ratio
TG	Triglycerides
TG/HDL	Triglyceride to high density lipoprotein ratio
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1. Country profile

Pakistan is a federal state, comprises of four provinces: Punjab, Sindh, Khyber pakhtunkwa, and Balochistan as well as the federally administered tribal areas, Azad Jammu and Kashmir, and Islamabad as Capital of the country. In 2009, Gilgit Baltistan was assigned a province like status to establish self-government. The majority of southern Pakistan's population lives along the Indus River while Karachi is the most populous city in Pakistan.¹



Figure 1: Map of Islamic republic of Pakistan

1.1.1. People and demography

The estimated population of Pakistan is over 187 million, and it is sixth most populous country in the world.² The population of Pakistan is distributed over four provinces; three fourth of population lives in rural areas, and forty three million are living below poverty line.³

Major ethnic groups constitute Punjabi, Sindhi, Baloch, Pathan and Mohajir (migrated from India at the time of division). Urdu is Pakistan's national language and it is commonly understood through out communities and considered as main language of communication. The prominent local languages are Punjabi, Sindhi, Saraiki, Pashto, Balochi, Hingko, Brahui, and Brushaski. English and Urdu are official languages at federal and provincial government setup. Majority (42%) of the workforce is linked with agriculture sector, other workforce areas are Services (38%) and Industry (20%).

During period of 1950 – 2011, the urban population of Pakistan increased by sevenfold whereas the increase in total population was fourfold. Currently the growth rate has been moderated to low i.e. 1.6% as compared to the previous high rate.⁴

1.1.2. Geography

The total area within the boundaries of Pakistan is 796,095 square kilometer. Gawader bay is the most southern part, and country extends northerly about 1,800 kilometers to the Khunjarab Pass going to border of China. Pakistan is located in southern part of Asia, with Arabian Sea in the south, China in north, Afghanistan and Iran in west and India in the east. The cost is 1,046 kilometers along Arabian Sea and land boundary is 6,774 kilometers with neighboring countries i.e. 909 kilometers with Iran, 2,430 kilometers with Afghanistan, 523 kilometers with China and 2,912 kilometers with India. There are three main geographic regions in Pakistan; mountainous northern region that includes i.e. Hindu Kush, Karakoram and Himalayas, sparsely populated Baluchistan and Plains of Punjab and Sindh. River

Indus is main river extending from Kashmir, traversing Punjab and Sindh, and ends at the Arabian sea.⁵

1.1.3. Economy

The economy of Pakistan is mainly dependant on agriculture sector while major bulk of export earning comes from textile industry. The transfer of money from overseas also contributes in establishing the economy.

There was a period of strong economic growth especially during period between 2003 to 2007. After 2007, the inflation has drastically affected the economy that was further deteriorated by 2010 floods. The disaster of 2010 flood affected all provinces and caused more then 43 billion US \$ damage to economy.⁶

Pakistan is provided with substantial natural resources like natural gas, coal, copper, iron, limestone, and salt. The major crops of Pakistan are wheat, rice, sugarcane, and cotton and fruits. In 2009, the GDP growth rate was 2.7% However, due to unequal distribution of resources and wealth, about one fifth of population has to survive on less then US \$ 1.25 per day.⁷

In 2011, the GDP (Gross Domestic Product) in Pakistan worth 211.09 billion US dollars.⁸

1.1.4. Education

The population with age more then 10 years has the literacy rate of 58.5%. Males has got higher literacy rate then females i.e. 70.2% and 46.3% respectively.⁹ The rate of literacy also varies by region and especially by gender e.g. the literacy rate is 3% in tribal areas.¹⁰

The government initiated a nation wide program in 1998 to counter illiteracy and to provide basic education to all children.¹¹ And it is expected that primary education will get 100% enrolment in year 2015 as ministry of education has applied various educational reforms for eradicating the illiteracy in the country.¹²

Table 1: Education infrastructure in Pakistan: ¹²

Education Centers	Total number
Primary Schools	155,000
Middle Schools	28,728
High Schools	16,100
Secondary Vocational Institutions	636
Arts & Science Colleges	1,066
Professional Colleges	382
Universities	51

1.1.5. Health profile

Provincial government regulates health matters; responsible for delivery and management of health services. However, federal government (Ministry of Health) decides about health policy, research, training, and seeking foreign assistance. The core of health system constitutes the Basic Health units (BHUs) and Rural Health centers (RHUs). The secondary care includes inpatient care facilities, provided through Tehsil headquarters' Hospitals (THQs) and District Health Quarter Hospitals (DHQs). Tertiary care via teaching hospitals is restricted to big cities of the country. Health sector in Pakistan got financing through taxation and out-of-pocket payments, however some minor contribution comes from donors. The social security schemes gives cover to only 3.5% employees, and 3.4% of population get support from Zakat and bait-ul-mal funds. In short, a comprehensive mechanism of social protection has not been developed.¹³

Table 2: Health infrastructure in Pakistan¹²

Hospitals	916
Dispensaries	4,600
Basic Health Units (BHUs)	5,301
Maternity & Child Health Centres	906
Rural Health Centres (RHCs)	552
Hospital Beds	99,908
Doctors (registered)	113,206
Dentists (registered)	6,127
Nurses (registered)	48,446
Paramedics	23,559
Lady Health Workers	6,741

1.1.6. Non-communicable diseases

Non- Communicable diseases are one of the major causes of mortality and morbidity among Pakistani adults and inflict a substantial economic burden on families, societies and health system.¹⁴ NCDs and injuries are among the top ten causes of morbidity and mortality in Pakistan, and they account for approximately 54.9% of total deaths.¹⁵

Usually, the workforce is the primary population that bears the burden of NCDs. In Pakistan, the current population-based morbidity data on NCDs showed that one in three adults above the age of 45 years is suffering from high blood pressure.¹⁶

In Pakistan, the diabetes is prevalent at the rate of 10%, whereas 40% men and 12.5% women use tobacco by chewing or smoking. Karachi has the highest incidence of breast cancer among Asian population. Moreover, the country bears the

burden of estimated one million severely mentally ill individuals, and 1.4 million road traffic accidents accounted for 7000 fatalities.¹⁷

Dealing with NCDs in Pakistan is a multidimensional challenge and requires appropriate investments and policies to be incorporated in development of health sector.¹⁸

1.2. Background

1.2.1. Diabetes mellitus

Diabetes mellitus is a metabolic disorder of various etiologies characterized by hyperglycemia due to insulin resistance and relative insulin deficiency.¹⁹

However, in other type of diabetes the pathology may differ e.g. in type 1 diabetes there is absolute deficiency of insulin due to autoimmune or idiopathic destruction of beta cell in pancreas. Type 2 diabetes constitutes 90% of cases with diabetes while remaining are primarily due to type 1 and gestational diabetes.²⁰

1.2.2. Global prevalence of diabetes

Up till 2011, there were 366 million people on the globe who had developed diabetes and it is estimated that 556 million will have diabetes in 2030.²⁷ This scenario creates a serious concern regarding the capabilities of these states to cope with economical burden they might face in future.

Table 3: Prevalence of diabetes and estimated diabetes numbers by region among adults age 20-70 years for the years 2011 – 2030.²⁷

	2011			2030			Increase
	Population (000s)	Cases (000s)	Adjusted prevalence (%) ^a	Population (000s)	Cases (000s)	Adjusted prevalence (%) ^a	
AFR	387	14.7	5	658	28	5.9	90.5
EUR	651	52.6	6	671	64	7.1	21.7
MENA	359	32.8	12.5	542	60	14.3	82.9
NAC	322	37.7	11.1	386	51.2	12.6	35.8
SACA	290	25.1	8.6	376	39.9	10.1	59
SEA	856	71.4	8.6	1188	120.9	10.5	69.3
WP	1544	131.9	10.1	1766	187.9	11.6	42.5
World	4409	366.2	8.3	5587	551.9	9.9	185.7

^a Age-standardized to the world population.

Throughout Asia, the proportion of people with diabetes and obesity has increased, and there are no sign of slowing down in the increasing rate. The most populous countries of the world are located here, and there have been major changes has occurred in demographic, socio-economic, and epidemiological status in recent decade. Additionally, the under estimation of the economic burden associated with type 2 diabetes in Asian region has contributed the delay in suitable prevention and management plans by national and regional government authorities.²²

In major south Asian countries, the prevalence of diabetes has increased drastically due to urbanization. For example in Bangladesh, the prevalence of diabetes in rural areas increase from 2.3% to the level of 6.8% in less then a decade.²³

In Pakistan, the prevalence of diabetes ranges from 7.6 to 11%.^{24,25,26}

1.2.3. Economic burden of diabetes

Globally, the prevalence of non communicable diseases is increasing remarkably. The deaths due to cardiovascular diseases have reached 18 million per year mainly contributed by diabetes and hypertension. The main culprit in this up rise of diabetes and hypertension is being identified as obesity. During the past decade, the developing world is threatened by joint venture of diabetes, hypertension, malnutrition and infectious diseases.²⁸

Increasing Prevalence of diabetes in developing countries, leading to increased morbidity and mortality.²⁹ Due to improvement in socio-economic conditions the lifestyle has changed and causing significant increase in population with obesity and diabetes.

1.2.4. Primary prevention of diabetes

Diabetes is preventable, however once developed, it becomes more difficult to prevent the progression and complication of the disease. Primary prevention of diabetes is under focus since last few decades and has given promising results, while

several approaches have been identified to achieve these positive outcomes. South Asian countries, constituting India, Pakistan, Bangladesh, Sri Lanka and Nepal, have emerged as the prime host for the epidemic of diabetes. This scenario has developed within a relatively short period of two to three decades.³⁰

For primary prevention trial, the risk score assessment of subjects to be screened has found cost effective.^{31,32} The screening benefits among south Asians would be high because a large population with undiagnosed diabetes and intermediate hyperglycemia lives here, and therefore a cost effective approach can be well anticipated by both the government and people.

In Pakistan, a primary prevention program³³ was conducted during the period of 2006 to 2009. Since December, 2011, a three year diabetes prevention program (PDPP) is under way in Karachi. This program was launched by Agha Khan University in collaboration by international Diabetes Federation and Helsinki University.

1.2.5. Diagnosis of diabetes

After every few years, the diabetes research communities reassess the current classifications, screening and diagnosis of diabetes, based on new research and clinical practices.

In 1997, the American Diabetes Association issued diagnostic criteria for diabetes, followed by update in 2003 and 2010.^{34,35} Currently, the diagnosis of diabetes is based on one of the four abnormalities: fasting blood glucose, random elevated glucose with symptoms, abnormal oral glucose tolerance test (OGTT), or hemoglobin A1C (HbA1C). The patients having impaired fasting glucose (IFG) and/or impaired glucose tolerance are considered as high risk individuals who can develop diabetes more often than normal individuals.

According to current ADA criteria, the diagnostic cut off for normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance and diabetes are as follows.^{36,37,38}

Normal — Fasting blood glucose (FBG) <100 mg/dL (5.6 mmol/L). Two-hour glucose during OGTT <140 mg/dL (7.8 mmol/L).

Categories of increased risk for diabetes

- Impaired fasting glucose (IFG) — FBG ≥100 to 125 mg/dL (5.6 to 6.9 mmol/L)
- Impaired glucose tolerance (IGT) — 2-h BG (75 g OGTT) ≥140 to 199 mg/dL (7.8 to 11.0 mmol/L)
- A1C — 5.7 to 6.4 percent (the International Expert Committee recommended 6.0 to 6.4 percent)

Diabetes mellitus —FBG ≥126 mg/dL (7.0 mmol/L), 2-h BG ≥200 mg/dL (11.1 mmol/L); random BG >200 mg/dL (11.1 mmol/L) in the presence of symptoms; A1C ≥6.5 percent

In absence of obvious symptoms of hyperglycemia, the diagnosis of diabetes must be confirmed by performing the same test on next day.

1.2.6. Screening of diabetes in developing countries

It is estimated that approximately 50% of all people with diabetes are not yet diagnosed. The diabetes that is undiagnosed found to be associated with higher risk of death. The Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study, that was conducted on approximately 25000 people for a mean follow up period of 7.3 years showed that the hazard ratio for death was twice for subjects with type 2 diabetes as compared to normal subjects diagnosed through case detection program. A similar, DECODA study conducted on Asian people reported similar trends.³⁹

In context of Asian countries, a major barrier towards population based screening of intermediate hyperglycemia is the expensive and time consuming OGTT.⁴⁰ Questionnaire based risk assessment of diabetes has been identified as an effective tool in European countries but such tools has not been validated in the developing

countries. The majority of people affected by IGT are living in middle or low income countries.⁴¹ Also the overall literacy level in developing countries may counter the effectiveness of such program.

American diabetes association (ADA) has recommended fasting blood glucose as the preferred test for diagnosing diabetes and prediabetes, the reason for this recommendation is based on ease of use, lower cost and acceptability to patients.⁴²

Also for clinical settings, the ADA expert panel has recommended the use of fasting blood glucose levels by doctors to screen their patients for diabetes because of ease and cost-effectiveness.⁴³

The fasting blood glucose values can easily be obtained at primary health care centers during routine visits and it can be performed with other test e.g. lipid profile that require a fasting blood sample. And because of the simplicity of getting the fasting blood glucose levels, it is expected that a greater number of diabetes cases who are not diagnosed can easily be detected in routine clinical settings as if compared with OGTT.⁴⁴

In health system perspective, screening is cost effective when compared to no-screening,⁴⁵ and this cost effectiveness seems apparent that if we are considering the FBG as a screening tool due to its lower cost. However, the low sensitivity of FBG testing may lead to high false negative results and can therefore indirectly influence the overall estimation of economic burden related to the management of prediabetes. On the other hand, OGTT has been widely accepted as gold standard for screening and diagnosis of diabetes, however the cost and the time duration required are the major drawback while performing the population-based screening of pre diabetes.

1.2.7. Intermediate hyperglycemia

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are the intermediate states between normal glucose hemostasis and diabetes. The abnormal glucose regulation associated with IFG and IGT is represented by blood glucose level cut offs at fasting (8-12 hour) and at 2 hours after meals. By definition, IFG is defined

as elevated plasma blood glucose level between 100 to 126 mg/dl, and impaired glucose tolerance (IGT) is defined as the 2- hour blood glucose ≥ 140 and < 200 mg/dl after 75 gram oral glucose load.⁴⁶

The mostly recommended sequence of testing for the diagnosis of diabetes is performing FBG first, and if found positive then on subsequent day followed by OGTT test to demonstrate presence of combined IFG/IGT.⁴⁷

When IFG was defined in 1997 by ADA as a means of classification of individuals having FBG between normal and diabetes, it was taken as analogous to IGT.⁴⁸ However, in 2003 the original FPG range (110 – 125) was modified to 100-125 mg/dl in order to make IFG testing more close to IGT test in identifying the population risk of developing diabetes. Consequently, the change in cut off point increased the prevalence of IFG by three to four folds in population.⁴⁷

The estimation of HbA1c has been included in the diagnostic criteria for diabetes since 2010 as recommended by ADA. In a systematic review⁴⁹ it was found, while referring to community and hospital based studies that the equivalent cut off points of HbA1c and FBG showed generally lower sensitivity in detecting IGT. In summary, HbA1c was found to have lower sensitivity but higher specificity than FBG to detect diabetes, however none of the two was found effective in detection of IGT. It was also interesting to note that both HbA1c and FPG do not include a glucose challenge.

For FBG to diagnose IGT, it's necessary for the person to be IFG first i.e. combined IFG-IGT, and therefore the complete inability of FBG to detect isolated IGT is obvious.

1.2.8. Insulin resistance

Insulin resistance can be defined as inability of a known quantity of endogenous or exogenous insulin to induce insulin action (glucose uptake and utilization) in an individual as much as it does in a normal person. Insulin action is defined as the consequences after binding of insulin with its plasma membrane receptor followed by a series of protein-protein interaction. Occurrence of insulin resistance is a part of cardio-metabolic abnormalities commonly referred as “Insulin resistance syndrome”

or “Metabolic syndrome”, and may lead to type 2 diabetes, atherosclerosis, hypertension or polycystic ovarian syndrome depends upon the individual’s genetic background.⁵⁰

1.2.9. Insulin resistance and intermediate hyperglycemia

Abnormalities in insulin action and insulin secretion are the major defects that are responsible for deterioration and progression of normal glucose tolerance to impaired glucose tolerance. It has been concluded by several prospective studies that insulin secretory dysfunction and insulin resistance predicts the transition of IGT to type 2 diabetes in different populations⁵¹ A study that was carried out among Pima Indians (native American Indians) has shown that in subjects with normal glucose tolerance, a low insulin secretory response (initial phase of insulin secretion) to an intravenous dose of glucose is an independent and additional tool to predict development of diabetes.⁵² The first phase of insulin secretion is the immediate release of pre-formed insulin from beta cells of pancreas in response to glucose load. The body tries to compensate the decrease in first phase insulin secretion by increase in insulin production leading to hyperinsulinemic state. This hyperinsulinemic state declines as the disease progress along with decay of pancreatic beta cells leading to overt diabetes.

The complications associated with IGT are mediated by glucotoxicity i.e. in excess, glucose directly damages protein, lipids and other molecules by non-enzymatic glycation. Glycation is a process of bonding of glucose with lipids or protein molecules. The accumulated advanced glycation end-products mediate the disease process by inhibiting the specific enzymatic function leading to micro vascular and macro vascular complications⁵³

There is a distinction exist between IGT and IFG with relation to insulin resistance. Several clinical studies have suggested that location of insulin resistance is different for these two disorders. The individuals with IGT have higher muscle insulin resistance along with mild hepatic insulin resistance, whereas the individuals with IFG have severe hepatic insulin resistance but their muscle insulin sensitivity is either normal or mildly deranged. The subjects with IGT also have abnormal or deficient

late-phase insulin secretion,⁵⁴ that is manifested by post prandial or after-meal hyperglycemia.

1.2.10. Insulin resistance and lipid abnormalities

Dyslipidemias and hyperinsulinemia are the distinctive findings in individuals with insulin resistance, and these derangements have been recognized as increasing risk for cardio metabolic disorders.⁵⁵ These Dyslipidemias show distinct pattern of altered lipid metabolism, particularly elevated plasma triglyceride and decreased HDL-cholesterol concentrations and the presence of small, dense LDL particles. The high triglyceride level in blood is associated with insulin resistance and obesity, and that is secondary due to high plasma insulin levels leading to increased esterification of fatty acids in liver resulting in formation of triglycerides. The low HDL level in blood is due to the increased catabolism of HDL molecules augmented by insulin resistance and hypertriglyceridemic states.⁵⁶

In obesity, the increase in size of adipose tissues result in increased flow of free fatty acids (FFA) to other tissue, and that is responsible for increase in triglyceride content of these tissues. This condition leads to development of insulin resistance accompanied by other adverse effects and referred as lipotoxicity. Also, due to the higher FFAs in blood followed by expansion in adipose tissues, there is impairment in ability of insulin to suppress hepatic glucose output. These higher FFAs in blood contribute in decreasing the uptake of glucose by skeletal muscles. The increased triglyceride content of muscles correlates directly with insulin resistance, while the FFAs content of muscle phospholipids correlates inversely with insulin sensitivity in skeletal muscles.⁵⁷

1.2.11. Serum lipid ratios as a marker of insulin resistance

The higher levels of LDL are atherogenic,⁵⁸ however high HDL levels in blood have found to be cardioprotective.⁵⁹ Higher concentration of TGs is also considered a risk factor for cardiovascular diseases. In addition to that, several studies are agreed

upon the utility of lipid ratios and found that TC/HDL and LDL/HDL are better predictor of cardiovascular diseases than individual lipid marker.^{60,61,62} A study has shown negative correlation between TC/HDL and insulin-mediated glucose disposal highlighting the utility of these ratios in determining the insulin resistance.⁶³

Applicability of TG/HDL in identify insulin resistance in Aborigines, Chinese, Europeans and immigrant South Asians was explored and found that the TG/HDL ratio may be a good marker to identify insulin-resistance.⁶⁴

In similar studies,^{65,66,67} the close association has been reported between triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL) and insulin resistance, and it has been recommended to use TG/HDL as the surrogates for insulin resistance.

In a study,⁶⁵ published in 2013, a strong association has been observed between TG/HDL ratio and insulin resistance in youths, and it was suggested that TG/HDL ratio can be a strong candidate for a surrogate of insulin resistance and atherosclerosis in young population.

1.3. Rationale of the study

The association of lipid ratios with insulin resistance among native south Asians has not been studied. The identification of insulin resistance by the routine estimation of fasting lipid profile and lipid ratios could be a way towards early identification of people at risk.

The link between insulin resistance and IGT has been established, however, in developing countries, clinicians may have subjected to use fasting blood glucose to diagnose diabetes as it is convenient and cost effective. But what if fasting blood glucose is found negative, it would be difficult to convince the person being tested that although he/she is normal but there might be a possibility of having isolated impaired glucose tolerance. Most likely the person who got negative result of fasting blood glucose may be asked to come after one year to check his/her blood sugar. And, therefore the population with isolated IGT would be ignored.

Fasting insulin levels and HOMA-IR has been identified as a surrogate for insulin resistance, but the cost and the acceptability of measuring insulin resistance via fasting insulin levels in routine clinical consultation needs to be considered. The laboratory cost for estimation of fasting insulin levels is three times higher than lipid profile test, and the acceptance and understanding of serum lipid measurement in general public is relatively high. Additionally, in a resource restricted country e.g. Pakistan, it would be worthwhile to investigate the utility of serum lipid ratios for identification of insulin resistance.

The purpose of this study is to find the predictability of FBG to diagnose IGT in our study population, and explore the association between serum lipid ratios and insulin resistance markers in IGT subjects.

1.4. Objective of the study

1.4.1. General objective

The major aim of study was to find the association between lipid ratios and insulin resistance in subjects with impaired glucose tolerance in Pakistan.

1.4.2. Specific objective

- 1- To examine the predictability of fasting blood glucose testing to identify impaired glucose tolerance (IGT) status in Pakistani population.
- 2- To compare and find the association between insulin resistance and lipid ratios in I-IGT and combined IGT-IFG group.
- 3- To explore the associated factors that can assist in early detection of impaired glucose tolerance.

1.4.3. Research question

- i. Is there any association between ratios of lipid profile components with insulin resistance and/or glycemic status among Pakistani subjects?
- ii. Is fasting blood glucose test sufficient to demarcate normal glucose tolerance from impaired glucose tolerance?
- iii. Can we suggest serum lipid ratios for screening of insulin resistant or impaired glucose tolerance status?

CHAPTER 2: METHODOLOGY

2.1. Research setting

Karachi: The data comprises of anthropometric and biochemical information of subjects living in Karachi. It is the largest city of Pakistan, fourth most populated city in the world⁶⁹ and also the capital city of the province of Sindh. Karachi is said to be the main industrial and trade center of the country, situated in southern end of Pakistan, it is the main sea-port dealing major bulk of import and export. Karachi is also called as mini-Pakistan due to several ethnic groups living here. Metropolitan of Karachi is divided in 5 districts; Karachi south, Karachi east, Karachi central, Karachi west, and Malir.⁷⁰

2.2. Study population

The study population comprised of Subjects from the primary prevention project that was conducted during period from 2006 to 2009 in Karachi and represents all the ethnic groups

2.3. Research design

This was a cross sectional study designed to explore the difference between sub-groups of intermediate hyperglycemia among Pakistani population

2.4. Criteria for inclusion

Inclusion of data at screening level was based on high risk identification for diabetes through questionnaire with following characteristics:

1. Age greater than 30 years
2. No known diabetes
3. At least one family member with diabetes
4. Absence of serious physical and mental disabilities
5. Willing to join the study

The data of all subjects with OGTT test, fasting lipid profile and fasting insulin levels was included in the study. Impaired glucose tolerance is described as blood glucose levels of ≥ 140 to < 200 mg per decilitre after twelve hour fasting with subsequent oral intake of 75 gram Glucose (Glaxose-D or dextrose). Testing was revised in subjects with abnormal results and the mean of the two tests was used to include subjects in the study.

2.5. Exclusion criteria

Non availability of required variables

2.6. Outcome assessment

The outcome of interest was prevalence of intermediate hyperglycemia in subgroups, association between lipid profile components, insulin levels, anthropometric measurements, fasting blood glucose and 2-hr blood glucose

2.7. Data collection

Data was taken from collaborative project of Baqai Institute of Diabetology & Endocrinology (BIDE), Diabetic Association of Pakistan (DAP), and University of Oslo (UiO). This project was aimed towards primary prevention of diabetes and extended over the period from 2006 to 2009. The data was collected from the project at screening level that constitutes the subjects with varying degree of glucose tolerance i.e. extending from normal glucose tolerance to diabetes based on OGTT test, and at baseline level that included anthropometric measurements and laboratory investigations.

The data was received as forms stored in lock and key designated for the project and located at Baqai Institute of Diabetology and Endocrinology (BIDE). The data information was then transformed into SPSS document for statistical analysis.

For the primary prevention project, around 1825 suspected high risk individuals identified through the 'high risk questionnaire' and from these identified high risk subjects 1739 underwent oral glucose tolerance test. The test was found positive for

747 individuals including IFG, combined IFG-IGT, I-IGT and diabetes. After excluding the normal and diabetic individuals, the data of 242 subjects was available for analysis with laboratory investigations (fasting blood glucose, 2-hour OGTT blood glucose, fasting lipid profile and fasting insulin levels).

2.8. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 20 software. Data are presented as means with standard deviation. The data was checked for normality. Comparisons of means between sub-groups of intermediate hyperglycemia (combined IFG-IGT and isolated IGT) were performed with one-way ANOVA. Linear correlations were estimated with Pearson correlations. Receiver operator curve characteristics were calculated while using the 75th percentile values of fasting insulin levels and HOMA-IR.

2.9. Variables

Anthropometric variables:

Age (years)

Gender

Body mass index

Waist to hip ratio

Systolic blood pressure (mm. Hg)

Diastolic blood pressure (mm. Hg)

Biochemical variables:

Fasting blood glucose (mg/dl)

2-hour blood glucose (mg/dl)

Total cholesterol (mg/dl)

Triglycerides (mg/dl)

High density lipoproteins (mg/dl)

Low density lipoproteins (mg/dl)

Fasting serum insulin levels (mg/dl)

TG/HDL ratio

TC/HDL ratio

LDL/HDL ratio

HOMA-IR

Age is defined as years, and the age of subjects included in the project was greater than 30 years. Anthropometric measurements included weight (Kg), height (cm), waist (cm) and hip circumference (cm). During the measurements the participants were asked to wear light clothing and take off their shoes. Height was measured to the nearest cm and weight to the nearest 0.1 kg. Standardized scale was used for measurement of weight and standardized stick was used for measuring height. Waist circumference was measured as central point between the iliac crest and lower margin of rib at mid axillary line.

Plasma glucose was measured by using the standard glucose oxidase-peroxide method while the lipid profile was estimated by standard enzymatic procedure. Fasting insulin levels was measured and used for calculating the insulin resistance by using fasting blood glucose

2.10. Sample size calculation

The minimum sample size calculated by using the following formula;

$$n = \frac{Z^2 pq}{\underline{\hspace{1cm}}}$$

$$d^2$$

Where

n = sample size

z = 1.96 at 95% confidence

Prevalence of impaired glucose tolerance = 15%

p= 0.15

q=1-p = 0.85

d = 0.05 maximum allowable error

$$n = \frac{(1.96)^2 \times 0.15 \times 0.85}{(0.05)^2}$$

$$n = 196$$

Total estimated sample size was 196.

2.11. Defining insulin resistance

There is not much consensus on defining the optimal cut off for insulin resistance. McAuley et al.⁷¹ concluded that fasting insulin > 12 mU/l in normoglycemic subjects is a remarkable specific test for insulin resistance. While Laassko⁷² has found that fasting insulin levels >18 mU/l as the indicator of insulin resistance in normal population. Both studies used euglycemic clamp (gold standard), however broad variation in cut off values was explained by difference in ethnicity.

Although considered as gold standard, euglycemic clamp technique may not be feasible in epidemiological studies due to cost and sophistication of the procedure.⁷³

Fasting insulin level is considered best available proxy to identify insulin resistance, however other simple measures are being sought because there are different methods of assaying insulin and therefore it is not possible to suggest a single universal cut-off. Insulin resistance could be defined as the 75th percentile of the fasting insulin level in non-diabetic population under study.⁷⁴ Similarly, insulin resistance can also be defined by 75th percentile of HOMA-IR in subjects without diabetes.⁷⁵ However in a study,⁷⁶ the 75th percentile of HOMA-IR has been used to define the cut off of insulin resistance in subjects with diabetes while concluding the

optimal cut offs for diagnosis of metabolic syndrome according to IDF and ATP-III criteria.

In a recent study⁷⁷ the insulin resistance has been defined by using the euglycemic clamp (gold standard), concluding that insulin resistance can be estimated with good sensitivity and specificity (89% and 65% respectively) from HOMA-IR values $2.8 < \text{HOMA-IR} < 5.8$ with HDL values being less than 51mg/dl.

In our study, the 75th percentile of fasting insulin level and HOMA-IR is being used considering our study population being non-diabetic.

2.12. Ethical clearance

The study protocol has been approved by the regional Norwegian Ethics Committee. The primary prevention project from where data was collected had approval from institutional review board of the Baqai Institute of Diabetology and Endocrinology (BIDE), and Norwegian research council.

CHAPTER 3: RESULTS

Primary prevention project:

Data cleaning

Data analysis
(SPSS version 20 for windows)

Screening data
N=1565

Variables:

- Fasting blood glucose
- 2-hours blood glucose

Frequencies of glycemic status among screened population

Sensitivity and specificity of Fasting blood glucose test

Baseline data
N=242

Variables:

- Age
- Gender
- Body mass index
- Waist hip ratio
- Systolic Blood pressure
- Diastolic blood pressure
- Total cholesterol
- Triglycerides
- LDL
- HDL
- Fasting insulin levels

Data splitting into two groups:

1. IGT
2. IFG-IGT

General Characteristics

- Anthropometric
- Biochemical

Correlation

ROC

Specificity & Sensitivity

Results are described in two separate sections. In first section, the screening data (N=1565) of the primary prevention project was analyzed considering two variables i.e. fasting blood glucose and 2-hour OGTT blood glucose. Afterwards, the sensitivity and specificity of fasting blood glucose with respect to oral glucose tolerance (OGTT) was estimated by cross tabulation.

In second section the baseline data of primary prevention project was analyzed (N=242), limited by the availability of required variables. The data was split into two groups i.e. subjects having both impaired fasting glucose and impaired glucose tolerance (combined IFG-IGT) and isolated impaired glucose tolerance (I-IGT), these groups were compared on the basis of mean differences and correlation. Afterwards, the Receiver operating characteristics (ROCs) were calculated for lipid ratios.

3.1. Analysis of screening data

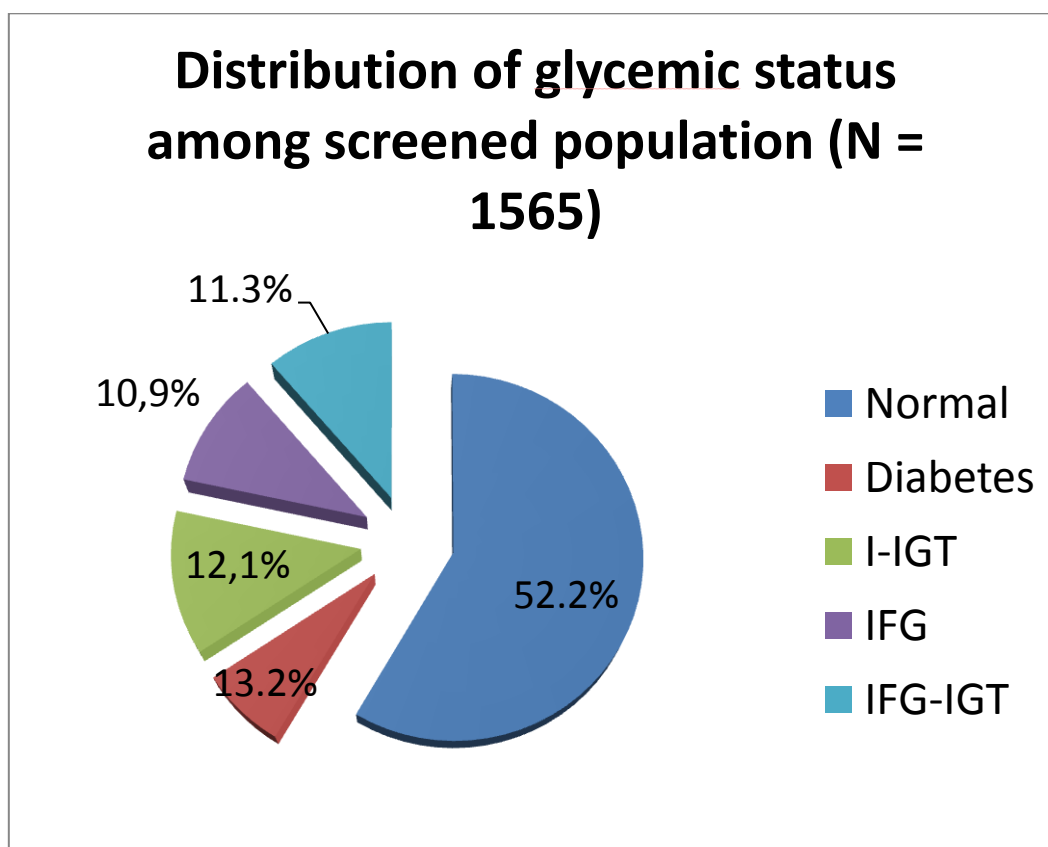


Figure 2: The distribution/frequency of glycemic status among the screened population

Figure 2 shows the distribution of glycemic status among the screened subjects. There were 818 (52.2%) subjects with normal glucose tolerance. The prevalence of prediabetes status as defined by the sub-groups i.e. IFG, combined IFG-IGT and I-IGT was 10.9%, 11.3% and 12.1% respectively. The diabetes was found to be 13.2% among the screened population.

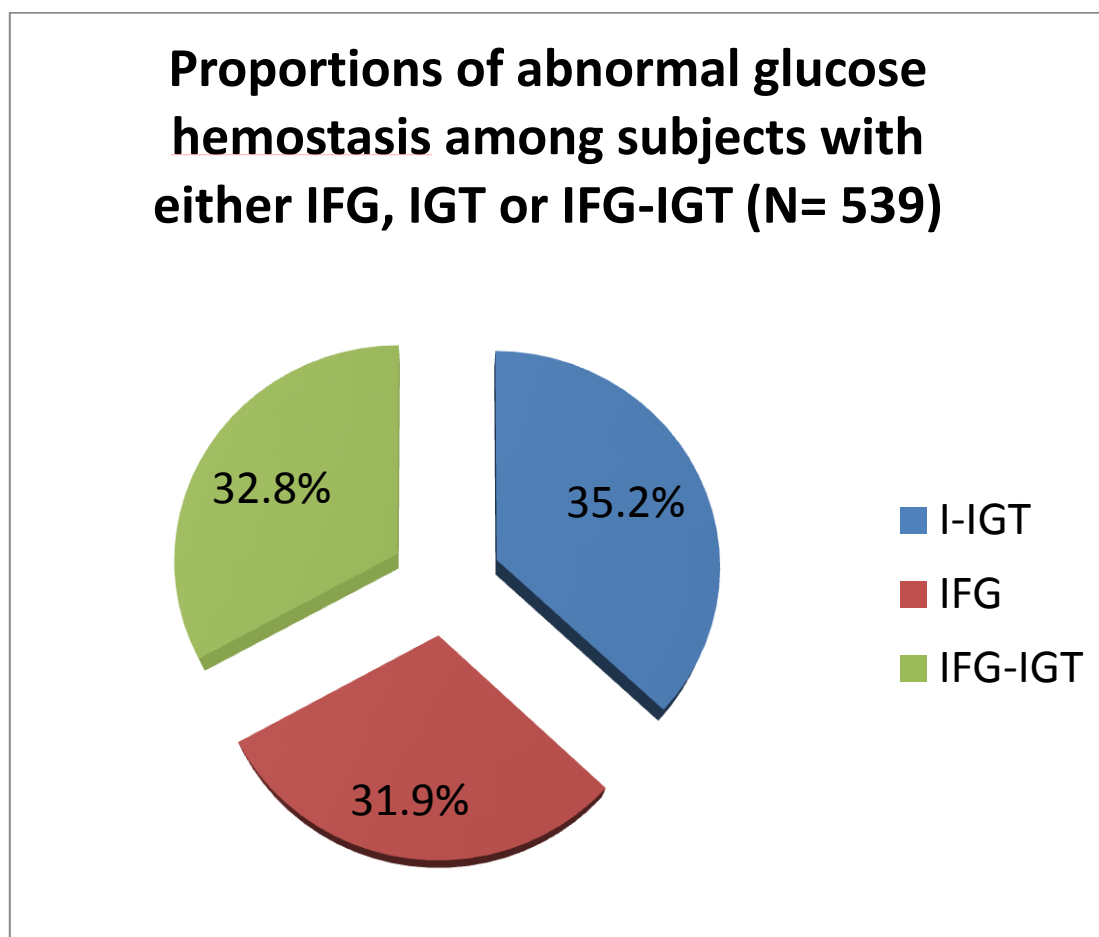


Figure 3: The distribution of intermediate hyperglycemia (prediabetes) in screened population

In figure 3, the percentages of prediabetes subgroups are shown. There were total 539 subjects found to have prediabetes and among them IFG, I-IGT, and combined IFG-IGT were 172 (31.9%), 190 (35.2%) and 177 (32.8) respectively.

Table 4: Cross tabulation between Fasting blood glucose levels (cut off points: ≥ 100 - ≤ 126) and 2-hour OGTT (≥ 140 - ≤ 200).

	IGT based on 2-hour blood glucose		
IFG	Positive	Negative	
Positive	177 (IGT-IFG)	172 (IFG)	349
Negative	190 (I-IGT)	818 (NGT)	1008
Total	367	990	1357*

*Excluding 208 diabetes cases

Table 5: The sensitivity and specificity of fasting blood glucose levels (cut off point ≥ 100 to < 126) to diagnose the impaired glucose tolerance (cut off

Sensitivity	48.2 %	95% CI: 43.0 % to 53.4 %
Specificity	82.6 %	95% CI: 80.1 % to 84.9 %
Positive Predictive Value	50.7 %	95% CI: 45.3 % to 56.0 %
Negative Predictive Value	81.1 %	95% CI: 78.6 % to 83.5 %

Table 4 shows the cross tabulation between fasting blood glucose with respect to OGTT in total 1357 subjects. The diabetes positive (208) cases were not included in the calculation. There were 177 true positive, 818 true negative, 172 false positive and 190 were false negative results. In table 5, the sensitivity and specificity of fasting blood glucose level was found to be 48.6% (95% CI: 43.5-53.8) and 82.6% (95% CI: 80.1-84.9) respectively. At the same time, the positive predictive value was 50.7% and negative predictive value was 81.1%.

3.2. Analysis of baseline data

3.2.1. General characteristics and comparison of groups

Table 6: General characteristics & comparison of means between combined IFG-IGT and I-IGT group

Variables	Combined IFG-IGT Mean \pm SD	I-IGT Mean \pm SD	Total Mean \pm SD	p-value
N	120	122	242	-
* Gender (Male/Female)	85/35	83/39	168/74	0.370
Age (years)	43.9 \pm 10.2	43.4 \pm 9.9	43.7 \pm 10.1	0.674
Body mass index (kg/m ²)	26.6 \pm 5.1	27.4 \pm 4.65	27.0 \pm 4.8	0.258
Waist to hip ratio	0.89 \pm 0.09	0.87 \pm 0.08	0.88 \pm 0.09	0.028
Systolic blood pressure (mm Hg)	120.8 \pm 14.1	119.7 \pm 17.20	120,2 \pm 15,8	0.621
Diastolic blood pressure (mm Hg)	83.1 \pm 12.1	83.8 \pm 9.92	83.5 \pm 11.0	0.602

* Categorical variable is expressed in number
p < 0.05 considered statistically significant

Table 6 shows the general characteristics of the subjects categorized by impaired glucose tolerance subgroups i.e. combined IFG-IGT and I-IGT, comprised of 168 man and 74 women with no significant difference(p>0.05) in gender ratio. The mean age of the subjects in combined IFG-IGT group was 43.9 \pm 10.2, and 43.4 \pm 9.9 in I-IGT group with no significant difference observed (p>0.05). Also, there were no

intergroup differences in Body mass index, Systolic blood pressure and diastolic blood pressure, however the waist to hip ratio was significantly higher in combined IFG-IGT group as compared to I-IGT group.

Table 7: Biochemical characteristics and comparison of mean between combined IFG-IGT and I-IGT group

Variables	Combined IFG +IGT Mean \pm SD	I-IGT Mean \pm SD	Total Mean \pm SD	p-value
N	120	122	242	-
OGTT-2 hrs blood sugar (mg/dl)	162.0 \pm 17.75	152.5 \pm 16.3	158.7 \pm 17.3	0.003
Total Cholesterol (mg/dl)	181.8 \pm 32.1	175.3 \pm 35.4	178.5 \pm 33.9	0.138
Triglyceride (mg/dl)	169.0 \pm 122.92	138.5 \pm 70.0	153.6 \pm 100.7	0.018
HDL (mg/dl)	37.9 \pm 6.3	38.2 \pm 4.6	38.1 \pm 5.5	0.679
LDL (mg/dl)	118.8 \pm 22.7	114.8 \pm 23.8	116.8 \pm 23.3	0.182
Fasting insulin levels (mpu/liter)	9.39 \pm 5.06	11.44 \pm 5.8	10.42 \pm 5.55	0.004

* p < 0.05 considered statistically significant

The biochemical characteristics of two groups (combined IFG-IGT and I-IGT) are shown in table 7. The mean 2-hour blood glucose (162.8 \pm 17.75) was significantly higher in combined IFG-IGT group as compared to mean 2-hour blood glucose (152.5 \pm 16.3) in I-IGT group. The lipid levels i.e. total cholesterol, high density lipoproteins, and low-density lipoproteins, however showed no significant difference

among the groups except the triglycerides levels that were significantly higher (169.0 ± 122.9) in combined IFG-IGT group when comparing the I-IGT group (138.5 ± 70.0). The mean fasting insulin level was also found significantly higher (11.44 ± 5.8) in I-IGT group as compared to combined IFG-IGT group (9.39 ± 5.06).

3.2.2. Correlations

Table 8: Correlation of blood glucose levels with fasting insulin levels in combined IFG-IGT and I-IGT group

Variables	Combined IFG-IGT		I-IGT	
	r	p-value	r	p-value
Fasting blood glucose	-0.313	0.001	0.214	0.020
2-hour blood glucose	-0.192	0.038	-0.032	0.730

p < 0.05 considered statistically significant

Table 8 shows the correlation between blood glucose levels with fasting insulin levels. In combined IFG-IGT subjects there was highly significant negative correlation ($r = -0.313$, $p = 0.001$) was found between fasting blood glucose and fasting insulin levels. There was also significant correlation exist between fasting blood glucose and fasting insulin levels in I-IGT group but the correlation was found to be positive ($r = 0.214$, $p = 0.020$). The 2-hour blood glucose in combined IFG-IGT group also showed positive correlation with fasting insulin levels, however there was no significance found among I-IGT subjects.

*Table 9: Correlation of serum lipids with fasting insulin levels among
Combined IFG-IGT and I-IGT group*

Variables	Combined IFG-IGT		I-IGT	
	r	p-value	r	p-value
TC	-0.275	0.003	0.133	0.153
TG	-0.169	0.071	0.254	0.006
LDL	-0.250	0.007	0.136	0.145
HDL	-0.131	0.164	-0.240	0.009

p < 0.05 considered statistically significant

TC = Total cholesterol

TG = Tryglycerides

LDL = Low density lipoproteins

HDL = High density lipoproteins

The association between individual serum lipid levels with fasting insulin levels is shown in table 9. In combined IFG-IGT group, the total cholesterol (TC) and low-density lipoproteins (LDL) found negatively correlated ($r = -0.275$ and $r = -0.250$) with fasting insulin level, however the triglycerides (TG) and high-density lipoproteins (HDL) showed no significant association among this group. Inversely, in I-IGT group, the TG and HDL showed significant correlation ($p = 0.006$ and $p = 0.009$) with fasting insulin levels, however, the TC and LDL did not show significant correlation with the fasting insulin levels.

Table 10: Correlation of serum lipids with HOMA-IR among combined IFG-IGT and I-IGT group

Variables	Combined IFG-IGT		I-IGT	
	r	p-value	r	p-value
TC	-0.269	0.004	0.125	0.177
TG	-0.169	0.072	0.214	0.020
LDL	-0.249	0.007	0.126	0.172
HDL	0.076	0.418	-0.236	0.010

*p < 0.05 considered statistically significant

TC = Total cholesterol

TG = Triglycerides

LDL = Low density lipoproteins

HDL = High density lipoproteins

In table 10, the correlation analysis is shown between surrogate of insulin resistance i.e HOMA-IR and the individual lipid profile component in combined IFG-IGT and I-IGT group. The TC and LDL were found negatively correlated ($r = -0.269$ and $r = -0.249$) with fasting insulin levels in combined IFG-IGT subjects, while TG and HDL showed no significant correlation in this group. Meanwhile the I-IGT group showed opposite results i.e. TG and HDL showed significant correlation with fasting insulin, however the correlation with TC and LDL was found insignificant.

Table 11: Correlation of lipid ratios with fasting insulin levels among combined IFG-IGT and I-IGT group

Variables	Combined IFG-IGT		I-IGT	
	r	p-value	r	p-value
TC/HDL	-0.142	0.129	0.282	0.011
TG/HDL	-0.133	0.155	0.359	0.001
LDL/HDL	-0.133	0.158	0.321	0.003

*p < 0.05 considered statistically significant

TC = Total cholesterol

TG = Triglycerides

LDL = Low density lipoproteins

HDL = High density lipoproteins

All the lipid ratios (TC/HDL, TG/HDL and LDL/HDL) showed no significant correlation ($P > 0.05$) with the fasting insulin levels in combined IFG-IGT group, however all the lipid ratios (TC/HDL, TG/HDL and LDL/HDL) were found significantly correlated with fasting insulin level in I-IGT group (Table 11).

Table 12: Correlation of lipid ratios with HOMA-IR

Variables	Combined IFG-IGT		I-IGT	
	r	p-value	r	p-value
TC/HDL	-0.140	0.134	0.272	0.014
TG/HDL	-0.135	0.150	0.339	0.002
LDL/HDL	-0.134	0.152	0.312	0.005

*p < 0.05 considered statistically significant

r = pearson's correlation

TC = Total cholesterol

TG = Triglycerides

LDL = Low density lipoproteins

HDL = High density lipoproteins

In table 12, the correlation of HOMA-IR with lipid ratios is shown. Although insignificant ($p>0.05$), all the lipid ratios ((TC/HDL, TG/HDL and LDL/HDL) showed negative correlation with HOMA-IR. While in I-IGT group, all the lipid ratios i.e TC/HDL, TG/HDL and LDL/HDL were found to have significant positive correlation ($p>0.005$) with the HOMA-IR index.

3.2.3. Receiver operating characteristics (ROC) analysis

Following section is related to the ROC analysis of lipid ratios (TG/HDL, TC/HDL and LDL/HDL) against the 75th percentile of the fasting insulin levels and HOMA-IR index. The 75th percentile of both the fasting insulin levels and HOMA-IR were used as cut off for insulin resistance in our study group and found to be 16.45 μ unit/liter and 3.8 respectively.

Triglyceride/High density lipoprotein ratio (TG/HDL):

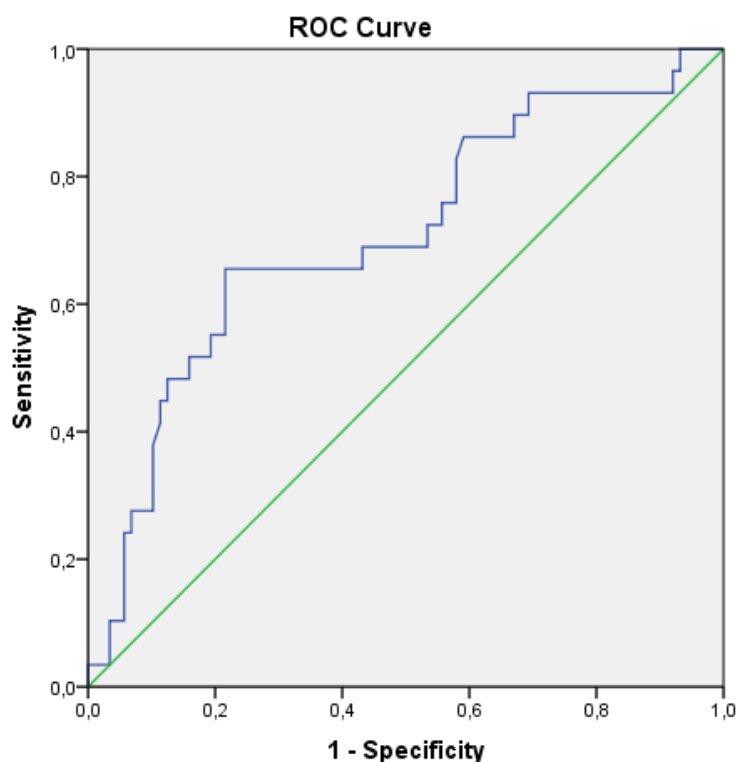


Figure 4: ROC curve of TG/HDL ratio with 75th percentile of fasting insulin level

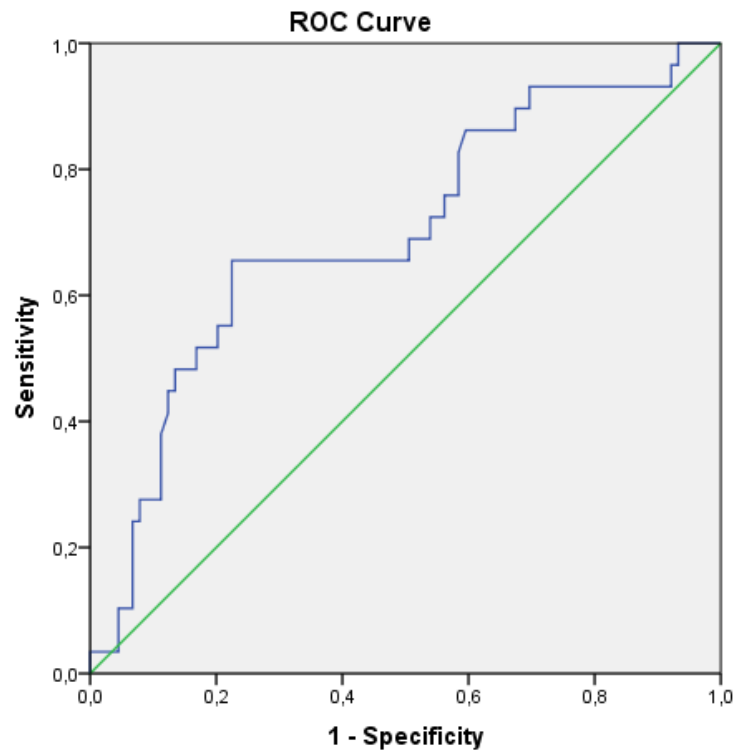


Figure 5: ROC curve of TG/HDL ratio with 75th percentile of HOMA-IR.

Figure 4 and 5 shows the ROC curve for the TG/HDL ratio with the 75th percentile of fasting insulin level (16.45) and HOMA-IR (3.8). The Area Under the Curve (AUC) for TG/HDL was found to be 0.707 and 0.697 for fasting insulin level and HOMA-IR respectively.

Total cholesterol/High density lipoprotein ratio (TC/HDL)

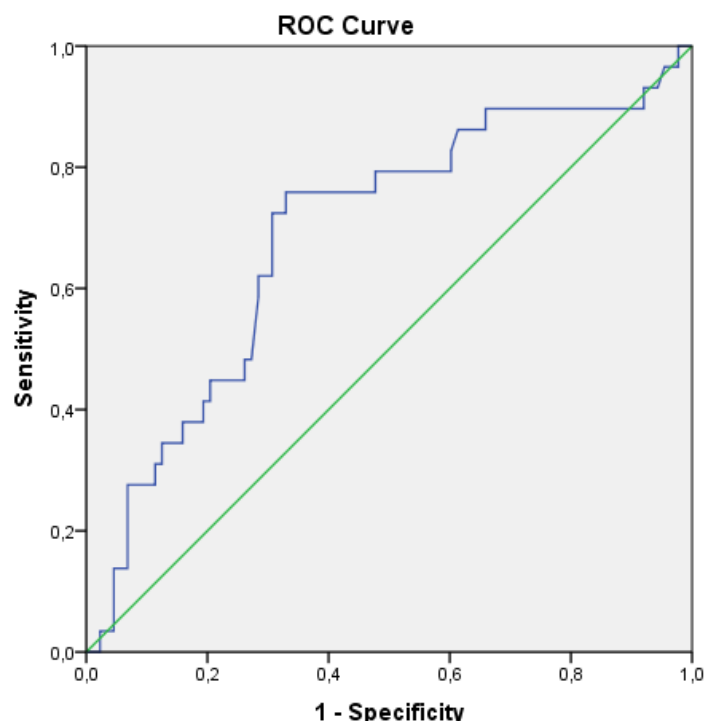


Figure 6: ROC curve of TC/HDL ratio (a) with 75th percentile of fasting insulin level

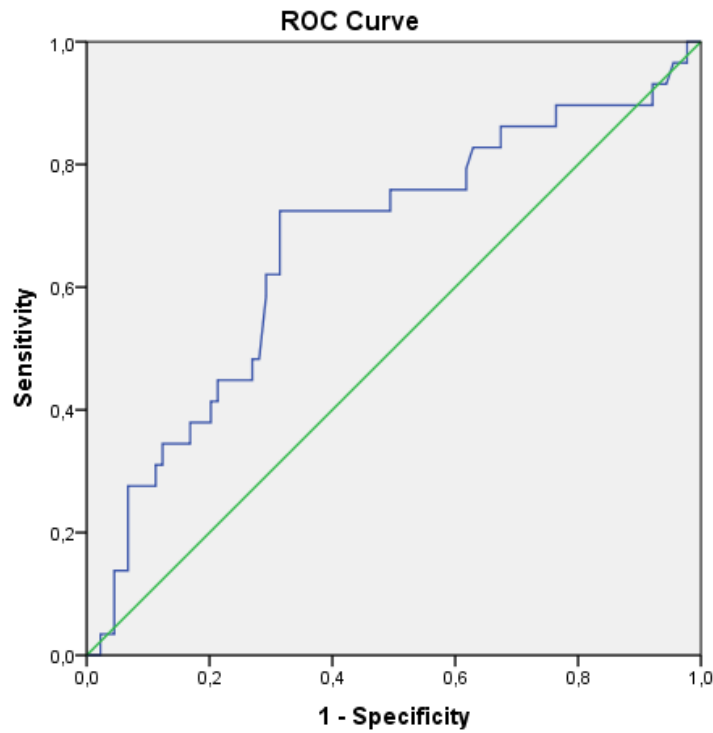


Figure 7: ROC curve of TC/HDL ratio with 75th percentile of HOMA-IR .

In figure 6, the ROC curve for TC/HDL ratio with 75th percentile of fasting insulin levels (16.45) is shown, having AUC of 0.688. The figure 7 shows the ROC curve plotted between TC/HDL and the HOMA-IR cut-off and AUC was found to be 0.668.

Total cholesterol/High density lipoprotein ratio (LDL/HDL):

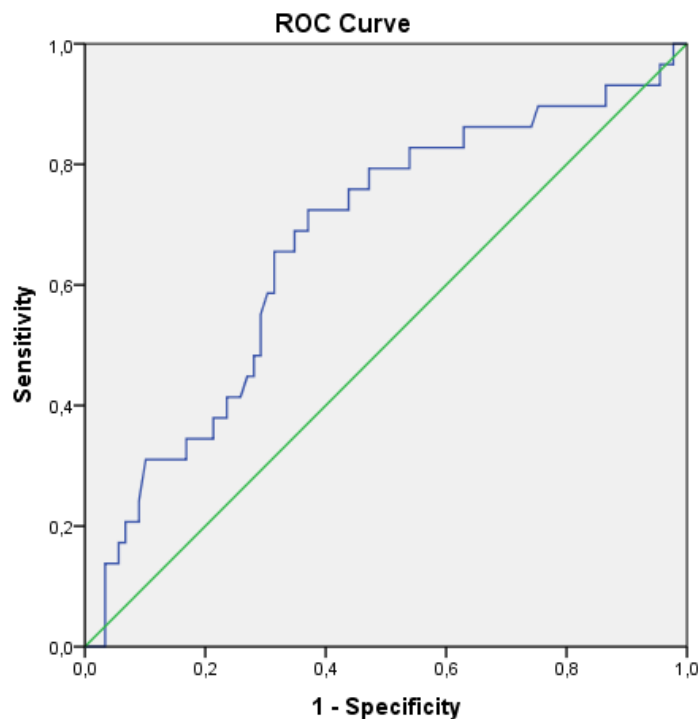


Figure 8: ROC curve of LDL/HDL ratio (a) with 75th percentile of fasting insulin level

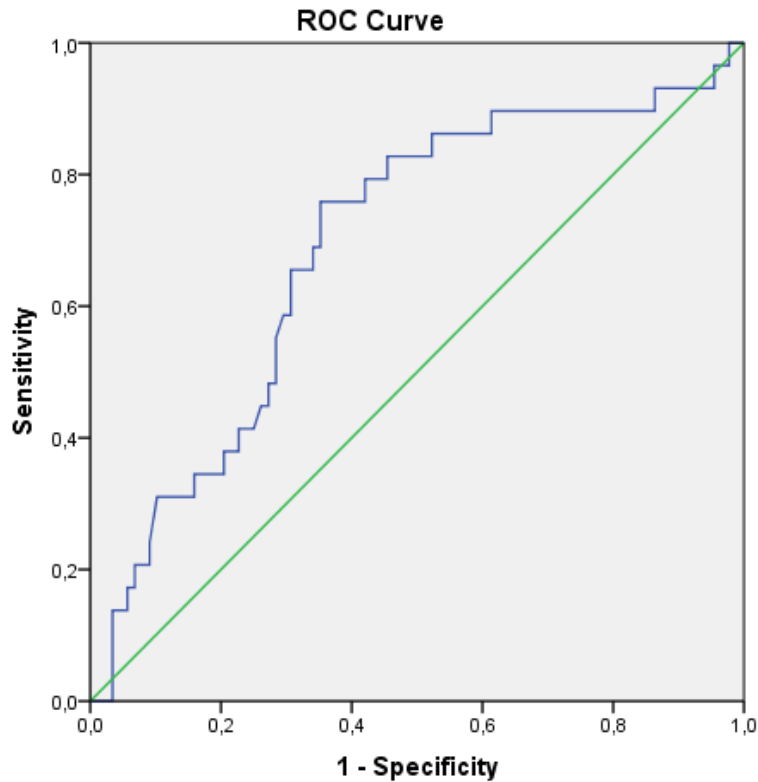


Figure 9: ROC curve of LDL/HDL ratio with 75th percentile of HOMA-IR.

The ROC curves of LDL/HDL ratio with insulin resistance cut offs is shown in figure 8 and 9. The AUC between LDL/HDL and 75th percentile of fasting insulin levels (16.45) was 0.690 and the AUC with 75th percentile of HOMA-IR (3.8) was found to be 0.670.

3.2.4. Sensitivity and specificity of lipid ratio with insulin resistance cut-off

The area under the ROC curve (AUC) is a summary statistic of diagnostic performance. The AUC could distinguish between non-predictive ($AUC < 0.5$), less predictive ($0.5 < AUC < 0.7$), moderately predictive ($0.7 < AUC < 0.9$), and highly predictive ($0.9 < AUC < 1$). The AUC of > 0.7 is considered moderately predictive and we have found AUC of 0.707 with TG/HDL ratio plotted against 75th percentile of fasting insulin level. Therefore, the sensitivity and specificity is measured and presented in table 10 with various cut offs of TG/HDL ratios.

Table 13: Lipid ratio (TG/HDL) values and their sensitivity & specificity with respect to 75th percentile fasting insulin level cutoff (16.45)

Lipid ratio (TG/HDL)	Sensitivity	Specificity
>1,6931	100%	6.8%
>2,2589	93.1%	20.5%
>2,3929	89.7%	30.7%
>2.6587	82.8%	42.0%
>2.8073	72.4%	46.6%
>2.9184	69.0%	50.0%
>3,0262	66.5%	56.8%
>3.3598	66.5%	70.0%
>3.7697	65.5%	78.4%
>4.0125	51.7%	80.7%
>4.8083	41.4%	88.6%
>5.1808	31.0%	89.8%
>6.6627	17.2%	94.3%
>8.7623	10.3%	96.9%
>11.3074	3.4%	100%

CHAPTER 4: DISCUSSION

The main objective of the study was to find the association between serum lipid ratios and insulin resistance surrogates. In our study we have found that all the lipid ratios (TC/HDL, TG/HDL, and LDL/HDL) in isolated IGT group were significantly associated with fasting insulin levels and HOMA-IR. In combined IFG-IGT group, however, there was no association observed between serum lipid ratios and insulin resistance. The presence of association between lipid ratios and insulin resistance in one subgroup of IGT signifies the importance of segregating and studying the I-IGT and combined IFG-IGT group separately. Clinically, it will help in targeting the insulin resistant population, a preferred domain for primary prevention of diabetes.

4.1. Methodological discussion

Cross sectional study may encounter a number of problems which may invalidate the results unless addressed properly. This methodology section has focused on objectives, however questions on strength and weaknesses of the study can be raised.

4.1.1. Strength of the study

4.1.1.1. Sample size

A large sample size was available for analysis that was otherwise difficult to obtain during the period for M Phil data collection. The greater the number of samples the higher would be the chances to find differences in mean. In first section of data analysis a sample size of 1565 subjects were available to generalize the results on the specific population with enough statistical power and avoiding the random error.

4.1.1.2. Cost and time

While doing the cross sectional data analysis, the prevalence of subgroups of intermediate hyperglycemia has been estimated by utilizing the current available data, that has minimized the cost and time. The prevalence of Isolated IGT has been

estimated first time in Pakistani population. Additionally, the associations were examined between anthropometric and biochemical variables within the same framework to extend the diversity of the study.

4.1.1.3. Experimentation

The cross sectional data analysis provides opportunity to accommodate new development in field of research, and therefore, redefining the categories and exploring the new objectives becomes possible. In our study, we are able to analyze the pre defined IGT group into two sub-groups i.e. Combined IFG-IGT and isolated IGT, and this segregation has provided the (could be) new explanation of insulin resistance and beta cell failure in these sub-groups.

4.1.2. Weaknesses of the study

4.1.2.1. Selection bias

Selection bias occurs when certain characteristics of the subject influence the inclusion, and consequently might also effect the outcome. The selection bias is mediated by inability to randomize, and if not accounted for or acknowledge, then the validity of the study is compromised. By using a random selection procedure we can control the selection bias by providing everyone the equal chance to be selected. Statistically, all observational studies have builtin bias but the challenge is to interpret how selection bias can effect the outcome of the research.

The two potential sources of selection bias has been identified for this study; the selection of samples during the initial phase of the primary prevention project, and during the acquisition and analysis of data for the current study. To identify the selection bias inharited with the parent project, it was sought how the randomization was done. The selection of subjects was based on OGTT results, this had made the selection specific to the high risk subjects. It was assured that all the subjects had similar baseline characteristics by stratification according to age. The measurement bias was addressed by using stuctured questionnaire, and availability of trained staff

to conduct measurement. For the current study, the data was acquired from the written forms and the inclusion of samples was based on the availability of all the variables required for the data analysis, thus 242 samples were available to perform the statistical analysis.

4.1.2.2. Confounding

When a part or entire variable is influenced by another variable, the issue is termed as confounding. It was addressed in parent project while doing the stratification by age during randomization. Majority of the data was collected from work force, while in Pakistan, the majority of the women are house bound. This may have limit the availability of large number of subjects who are relatively less active. However, the representation of gender, age and BMI in groups were similar to minimize the confounding effect.

4.1.2.3. External validity for generalization

External validity for generalization is assured when results of a study can be generalized to other situations and to other people in a population. In our study the samples were gathered from Karachi which have proportions of all the major ethnic groups of Pakistan, and efforts were made that sample would represent the general population. The selected high risk population based on IGT is comparable to the same high risk population in Pakistan.

4.1.2.4. Cause and effect relationship

Although we have found significant association between the lipid ratios, fasting insulin levels and HOMA-IR, the inability of cross sectional data analysis to define cause and effect differentiation has limit the significance of the study.

4.1.2.5. Limited variables

A very specific weakness related to the study is unavailability of IFG (subgroup of intermediate hyperglycemia) to explain the results more precisely. Although, studies

are available that has analyzed the three subgroups of intermediate hyperglycemic (I-IFG, combined IFG-IGT, I-IGT) providing the reason for analyzing two groups, the completeness of analysis can be questioned. However, this weakness can provide basis for future study addressing all subgroups.

4.1.2.6. Weaknesses related to primary prevention project

Accumulations of weaknesses related to the primary prevention project are inherited and unadjustable i.e. related to the methodology (survey techniques, anthropometric & biochemical measurements, and selection biases).

4.2. Discussion on findings

4.2.1. *Prevalence of sub-groups of intermediate hyperglycemia*

In Pakistan, the studies done on intermediate hyperglycemia has followed the definition of IGT consisting of both I-IGT and combined IFG-IGT, this could have led the non availability of any study comparing I-IGT and combined IFG-IGT group in Pakistani population. In this study, we have found the opportunity to find out the prevalence of isolated IGT first time in study population.

The overall prevalence of intermediate hyperglycemia in our study population was 36.3%, constituting the I-IFG, combined IFG-IGT and I-IGT in proportion of 10.9%, 13.2% and 12.1% respectively. The relatively higher prevalence of intermediate hyperglycemia in our study as compared to previous studies^{81,82,83,84} conducted in four phases in four provinces by National diabetes survey of Pakistan, and in another study by Hydrie et al.⁸⁵ is probably due to the selection of high risk subjects (family history of diabetes and age > 30 years).

The importance of segregating the intermediate hyperglycemia into the respective subgroups has provided opportunity to examine the differences in anthropometric and biochemical parameters. A similar study being done recently in Bangladesh⁸⁶ showed the prevalence of I-IFG, combined IFG/IGT and I-IGT being 1.3%, 2.3% and

2.3%. Again, the low prevalence of intermediate hyperglycemia as compared to our study is most probably due to the selection of general rural population irrespective of risk factors, also the lower age limit (>20 years) may have to be considered as a cause of relatively low prevalence.

4.2.2. Predictability of fasting blood glucose to diagnose impaired glucose tolerance

In our study, the fasting blood glucose level (cut off point ≥ 100 to < 126) was found to have sensitivity of 48.2% to identify IGT subjects in study population. In a similar study⁷⁸ that was conducted to evaluate the diagnostic predictability of fasting blood glucose cut off with respect to OGTT (gold standard), it was found that if fasting blood glucose cut-off would have been used alone, 81% of subjects with isolated IGT would be diagnosed as having normal glucose tolerance. A similar study⁷⁹ has concluded that screening for diabetes using fasting glucose levels had very low sensitivity as compared to OGTT.

The low sensitivity of FBG in detecting IGT signifies the importance of exploring differences among the subgroups of intermediate hyperglycemia. According to the existing classification, impaired glucose tolerance comprises both isolated and combined IFG-IGT group, the further subdivision of IGT has been explored in our study to identify differences among the subgroups. As suggested by a study⁸⁰ the isolated IGT and combined IFG-IGT follow different pathways towards development of diabetes, same pattern is seen in our study population.

4.2.3. Differences between combined IFG-IGT and Isolated IGT group

There were significant differences found between the combined IFG-IGT group and I-IGT group, these differences were observed in waist to hip ratio, OGTT 2 hour blood glucose, triglycerides and fasting insulin levels. According to a five year follow-up study conducted in Danish population i.e. INTER99⁸⁰ it was concluded that IFG, isolated IGT and combined IFG-IGT are distinct pre-diabetes status and follow a different pathway towards development of diabetes. In the same study it was demonstrated that early compensatory increase in beta-cell function may be initiated by higher elevated fasting plasma glucose (IFG and combined IFG-IGT) but not by 2

hour plasma blood glucose level. It was also observed that the characteristics of combined IFG-IGT subjects are more in common with IFG subjects as compared to I-IGT subjects. It was therefore suggested that the subjects with isolated high 2-hour blood glucose should be titled as different entity from combined IFG-IGT as it is currently suggested by World Health Organization.

No gender and age difference among the group were found and hence provided the lesser confounding owing to these variables. The BMI wasn't differ between the group and i.e. already high according to the classification of current normal BMI in Asian population.

The difference in anthropometric measurement among the groups included waist to hip ratio that was found significantly higher in the combined IFG-IGT group, the biochemical parameters i.e. 2-hr blood glucose and triglycerides were also significantly higher in the combined IFG-IGT group. Higher waist to hip ratio, 2 hr blood glucose and triglycerides represents the more deteriorated metabolic status among combined IFG-IGT group, however the significantly higher insulin levels in I-IGT group represents a distinct feature representing the presence of insulin resistance in this group.

Several studies^{87,88,89} has examined the ADA defined IGT⁹⁰ as separate IFG-IGT and I-IGT groups. This subdivision in our study has led to some important findings related to the associated markers of insulin resistance and beta-cell failure.

4.2.4. Correlation of fasting insulin levels with blood glucose levels

This section includes the discussion on correlations between insulin resistance markers (fasting insulin levels and HOMA-IR) and variables in both I-IGT and combined IFG-IGT group. Perhaps the most interesting finding is the presence of significant positive correlation of fasting insulin levels with fasting blood glucose in I-IGT group, and significant negative correlation of fasting insulin levels with fasting blood glucose in combined IFG-IGT group. It has been established in various studies^{71,72,91} that insulin resistance is characterized by the high insulin levels, and at the same time the progression of intermediate hyperglycemia is represented by

higher blood glucose levels. Therefore, the reciprocity exist between fasting insulin levels and fasting blood glucose in combined IFG-IGT group indicates that progression/worsening of combined IFG-IGT status is associated with beta cell failure i.e. characterized by decline in fasting insulin levels. Where as in I-IGT group, there is significant positive correlation is found between fasting insulin levels and fasting blood glucose, indicating the increment in insulin levels with progression/worsening of I-IGT status. It is therefore suggested that the basic pathophysiological mechanism behind progression/worsening of I-IGT status is insulin resistance.

The association between fasting insulin levels and 2-hour blood glucose was significant in combined IFG-IGT group, however no significance between these two was observed in I-IGT group. While describing the association between fasting insulin levels and 2-hour blood glucose, it is important to notify that glycemic response needs to be assessed by insulin levels at that particular instance i.e. at 2-hr, and in our study, only the fasting insulin levels were available. Therefore, the associations between fasting insulin levels and 2-hour blood glucose could not be explained on the basis of current result.

4.2.5. Correlation between insulin resistance surrogates with serum lipids

We have examined the association between serum lipids and their ratios with fasting insulin levels and HOMA-IR. The derangement in serum lipids is one of the characteristics of intermediate hyperglycemia,⁹² and our results are consistent with this observation. In examining the association, we have found interesting results i.e. in combined IFG-IGT group, the serum total cholesterol and LDL were negatively associated with fasting insulin levels, probably due to the declining insulin levels associated with beta cell failure,⁹³ however, the HDL and TG showed no association with fasting insulin levels in combined IFG-IGT group. The association of HDL and TG with insulin resistance has been found independently in several studies^{94,95,96} and absence of such association (in our study) confining to the combined IFG-IGT group may have indicate that insulin resistance is not a characteristic of this group. However, the significant association of fasting insulin levels with TC and LDL may have indicated that intermediate hyperglycemia as single entity is generally

associated with dyslipidemias, but more precise explanation is needed to describe the finding.

The I-IGT group has shown association of fasting insulin levels with serum TG and HDL, probably indicating the peculiar pattern (high TG low HDL) of dyslipidemia in this group. As mentioned earlier, the high TG and low HDL are characteristic of insulin resistance, this finding has further added the assumption that insulin resistance could be the pathophysiological mechanism behind the development of I-IGT, however as a rule, the causality could not be inferred on the basis of cross sectional analysis.

4.2.6. Lipid ratios and insulin resistance

An apparent distinction is observed between the two groups i.e. significant positive correlation was found between all the lipid ratios (TC/HDL, TG/HDL, and LDL/HDL) and fasting insulin levels among I-IGT group and no significant observation was noted for combined IFG-IGT group. Similar results were observed while comparing the groups by using the HOMA-IR as surrogate for insulin resistance. In a previous study⁹⁷ conducted in Canada, that included four ethnicities including immigrant south Asians, has found lipid ratios to be associated with insulin resistance in all ethnicities except in immigrant south Asians. The reason could be the use of different BMI cut off for south Asians. Among all lipid parameters and ratios, the TG/HDL ratios has consistently been under consideration due to better association with insulin resistance.^{68,98,99} Similarly, In our study, TG/HDL ratios had the highest correlation with fasting insulin level and HOMA-IR ($r = 0.359$, and $r = 0.339$) in I-IGT group. However, the insignificant but negative correlation with IR surrogates in combined IFG-IGT group is showing same trends i.e. observed for individual lipid components and fasting blood glucose levels.

4.2.7. Sensitivity and specificity of TG/HDL ratio with insulin resistance cut offs

The sensitivity and specificity of serum lipid ratio has been estimated with the insulin resistance cut offs. Considering the AUC > 0.7 as moderately predictive, the TG/HDL ratio shown to have the reasonable specificity and sensitivity at various cut offs. With

the ratio of > 3.36 , the TG/HDL ratio has shown the sensitivity and specificity of 65.5% and 70% respectively. The observed value of TG/HDL ratio in our study is consistent with McLaughlin and colleagues⁶⁸ who have found that TG/HDL ratio ≥ 3.5 has a moderate ability to predict insulin resistance (47% sensitivity, 88% specificity) in Caucasian population. However, in a similar study¹⁰⁰ done on non Hispanic Caucasians and Mexican Americans, it was reported that the optimal cut off point of hyperinsulinemia (insulin resistance) was 3.0. The same study also reported that a single cut off point may not be applicable to a population with different ethnic groups.

In a recently conducted study¹⁰¹ on patient with PCOs (polycystic ovarian disease) has found strong correlation between serum lipid ratios (TC/HDL, TG/HDL and LDL/HDL) and insulin resistance. PCO is a classical example of insulin resistant status, thus similar trends in our study, indicates potential utility of these ratios in I-IGT group.

CHAPTER 5: CONCLUSION, RECOMMENDATIONS AND FUTURE RESEARCH IMPLICATION

5.1. Conclusion

We have found significant correlation between serum lipid ratios (TC/HDL, TG/HDL and LDL/HDL) and surrogate markers of insulin resistance in isolated IGT group. The highest correlation was observed between TG/HDL and fasting insulin levels. The AUC for the various cut offs of TG/HDL ratio was measured against the 75th percentile of insulin resistance surrogates, and it was found that lipid ratios, specifically TG/HDL ratio can be proposed has a potential marker for insulin resistance in Pakistani population.

The individual serum lipid component (TC, TG, LDL and HDL) and their ratios were found to have significant and opposite correlations with fasting insulin levels and HOMA-IR among IGT sub groups. Also, the significantly higher insulin levels in isolated IGT may have indicated that insulin resistance could be the characteristics of this group. The findings in our study may suggest that the IGT group as a whole can be sub divided into combined IFG-IGT group and isolated IGT group to explore the differences that may lead to the better understanding of pathophysiology of impaired glucose tolerance.

Due to the weak predictability of fasting blood glucose to diagnose IGT status, it is therefore suggested to use better option for the diagnosis of IGT in clinical practice. However, serum lipid ratios could be a potential option if used in conjunction of fasting blood glucose to identify subjects with isolated IGT who are found significantly more insulin resistant, and thus, can be more benefited by primary prevention of diabetes.

5.2. Recommendations

- Diagnosis of intermediate hyperglycemia is of utmost importance in primary prevention of diabetes, and only the execution of such program if based on fasting blood glucose can results in a high proportion of IGT individuals remaining undetected. The target population needs to be identified that can

better be benefited by the resource restricted intervention efforts in developing countries.

- OGTT would be the best screening tool, however the other convenient and cost effective methods needs to be explored to extend the primary prevention efforts in clinical practices
- Reliance on FBG on screening of diabetes in clinical practice needs to be reassessed.
- Combined testing using fasting blood glucose and fasting lipid profile needs to be explored in context of screening for intermediate hyperglycemia.

5.3. Future research implications

- Further research is needed to explore the implication of FBG and lipid profile in identifying intermediate hyperglycemia. The overall acceptance of lipid profile testing in community could be helpful to validate the findings by involvement of a large number of populations.
- Isolated IGT group can present the research ground for investigating the phenomenon of insulin resistance by more sophisticated methods i.e. euglycemic clamp in Pakistani population.

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Appendix I



REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Tor Even Svanes	22845521	02.07.2012	2012/952
			Deres dato:	Deres referanse:
			22.05.2012	

Vår referanse må oppgis ved alle henvendelser

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0317 Oslo

2012/952 Insulin resistance and lipid profile; trends in sequelae of impaired glucose tolerance among subjects from Karachi, Pakistan

Forskningsansvarlig: Universitetet i Oslo
Prosjektleder: Akhtar Hussain

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 14.06.2012. Vurderingen er gjort med hjemmel i helseforskningsloven § 10, jf. forskningsetikklovens § 4.

Prosjektomtale

The purpose of this study is to observe the sequential trends of pre-diabetes defined by IGT in progression to diabetes or reversal to normal glucose tolerance over the period of eighteen month, and to observe the effect of change in status of IGT on insulin resistance and lipid profile in our population. The earliest detectable abnormality in type 2 diabetes is impairment in the body's ability to respond to insulin. Applicability of TG/HDL-C in identify insulin resistance in various ethnic groups was explored and found that the TG/HDL-C ratio may be a good marker to identify insulin-resistance. Further studies needs to be targeted towards exploring pattern of atherogenic dyslipidemias with relation to changing glyceemic status and insulin resistance over the time, and role of dyslipidemias in predicting insulin resistance and diabetes in target population. The study is retrospective, and data will be taken from collaborative project of Baqai Institute of Diabetology and Endocrinology (BIDE), Diabetic Association of Pakistan (DAP) and the University of Oslo. This project was aimed towards primary prevention of diabetes and extended over the period from 2006 to 2009.

Vurdering

Komiteen opplever dette som et nyttig prosjekt, og legger til grunn at det også avklares og innhentes etisk godkjenning fra de rette institusjoner i Pakistan.

Vedtak

Prosjektet godkjennes, jf. helseforskningslovens § 9 og 33.

I tillegg til vilkår som fremgår av dette vedtaket, er tillatelsen gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden og protokollen, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Tillatelsen gjelder til 31.12.2013. Av dokumentasjonshensyn skal opplysningene likevel bevares inntil 31.12.2018. Opplysningene skal lagres avidentifisert, dvs. atskilt i en nøkkel- og en opplysningsfil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato.

Besøksadresse:
Gullhaug torg 4A, Nydalen,
0484 Oslo

Telefon: 22845511
E-post: post@helseforskning.etikk.no
Web: <http://helseforskning.etikk.no/>

All post og e-post som inngår i
saksbehandlingen, bes adressert til
REK sør-øst og ikke til enkelte
personer

Kindly address all mail and e-mails
to the Regional Ethics Committee,
REK sør-øst, not to individual staff
persons

Prosjektet skal sende sluttmelding på eget skjema, jf. helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Komiteens avgjørelse var enstemmig. Prosjektleder skal sende søknad om prosjektendring til REK sør-øst dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. helseforskningsloven § 11.

Klageadgang

Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

Arvid Heiberg
prof. dr.med
leder REK sør-øst C

Tor Even Svanes
seniorrådgiver

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